

WHAT IS CLAIMED IS:

1. A method for the identification of aberrant phenotypes expressed by neoplastic cells comprising the steps of:

a) separately staining one or more normal/reactive samples and one neoplastic sample with overlapping multiple combinations of monoclonal antibodies, each monoclonal antibody in each combination being conjugated to a different fluorochrome and each combination having in common at least three fluorochrome conjugated monoclonal antibodies;

b) sequentially measuring the fluorescence emissions associated to large numbers of cells stained with each of the combinations of monoclonal antibodies from the normal/reactive samples and the tumor sample, using flow cytometry;

c) storing two independent list mode data files, one containing information on the specific light scatter and fluorescence characteristics of each cell analyzed from the normal/reactive samples and the other containing information on the specific light scatter and fluorescence characteristics of each cell analyzed from the neoplastic sample;

d) creating new data files by merging, at known proportions cellular events from the data file containing information about the cells present in the neoplastic sample into the data file containing information on the cells present in the normal/reactive samples;

e) defining in a multidimensional space generated by the flow cytometric measurements of light scatter and fluorescence emissions, those areas occupied by events corresponding to normal cells and those areas corresponding to empty spaces in normal/reactive samples and that may be occupied by tumor cells in neoplastic samples;

f) sequentially identifying in the data files merged as described in step e), those events corresponding to neoplastic cells as different from the events corresponding to normal cells coexisting in a multidimensional space generated by the flow cytometric measurements of light scatter and fluorescence emissions; and

g) establishing the most relevant phenotypic aberrations displayed by the neoplastic cells as compared to their normal counterpart, that allow their unequivocal, sensitive and specific identification in the merged data file.

2. The method of claim 1, wherein the samples comprise peripheral blood.

3. The method of claim 1, wherein the samples comprise bone marrow.

4. The method of claim 1, wherein the samples comprise spinal fluid.

5. The method of claim 1, wherein the samples comprise lymph node.

6. The method of claim 1, wherein more than one randomly selected normal sample is stained.

7. The method of claim 1, wherein more than one normal sample is stained, all normal samples being selected from a well defined age group of individuals, or from any other group of individuals defined according to their gender and underlying non-neoplastic conditions.

8. The method of claim 1, wherein the neoplastic samples contain hematopoietic tumor cells of one or more different types.

9. The method of claim 1, wherein the neoplastic samples contain non-hematopoietic tumor cells of one or more different types.

10. The method of claim 1, wherein the neoplastic samples contain both hematopoietic neoplastic cells and non-hematopoietic tumor cells.

11. The method of claim 1, wherein the neoplastic samples are obtained at first diagnosis, relapse and at any time period after diagnosis.

12. The method of claim 1, wherein the neoplastic samples may contain high or minimal numbers of neoplastic cells.

13. The method of claims 1, wherein the samples are stained directly after being obtained.

14. The method of claim 1, wherein the samples are stained after being cultured in vitro.

15. The method of claim 1, wherein the panels of multiple combinations of monoclonal antibodies used to stain neoplastic samples and normal/reactive samples are identical.

16. The method of claim 1, wherein the panel of multiple combinations of monoclonal antibodies used to stain neoplastic samples are shorter than the panel of combinations of monoclonal antibodies used to stain normal/reactive samples, but the former panel is fully contained in the latter one.

17. The method of claim 1, wherein for each pair of panels of combinations of monoclonal antibodies, an exact clone of each monoclonal antibody used in each individual combination of monoclonal antibodies, and a fluorochrome to which it is conjugated, are identical in the two panels of combinations of monoclonal antibodies.

18. The method of claim 1, wherein the number of monoclonal antibodies contained in each combination is composed of four or more different monoclonal antibody reagents.

19. The method of claim 1, wherein the number of monoclonal antibody reagents in common for all said combinations, is of three or more different monoclonal antibodies.

20. The method of claim 1, wherein the exact monoclonal antibodies that are common to all combinations of monoclonal antibodies used in a panel to stain a pair of normal/reactive samples and a neoplastic sample may vary depending on the type, the lineage and the maturation stage of the tumor cells contained in the neoplastic sample.

21. The method of claim 1, wherein at least four different fluorochromes are used, each being conjugated to a different monoclonal antibody, a fluorescence emission of each fluorochrome being distinguishable from that of the other fluorochrome-conjugated monoclonal antibodies.

22. The method of claim 21, wherein a combination of compatible fluorochromes is selected from fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridin chlorophyll protein (PerCP), allophycocyanin, alexa fluor 488, alexa 647, pacific blue, alexa fluor 405, cyanin 5 (Cy5), cyanin5.5(Cy5.5) and conjugates thereof coupled to PE, to APC or to PerCP (PE/Cy5, PE/Cy5.5, PE/Cy7, APC/Cy7 and PerCP/Cy5.5) or any additional compatible fluorochrome or fluorochrome-conjugates.

23. The method of claim 1, wherein information on the light scatter and the fluorescence measures of those

events stained with each combination of monoclonal antibodies conjugated to fluorochromes contained in a panel used to stain different replicate aliquots of the same sample are initially stored in separate data files.

24. The method of claim 1, wherein information on the light scatter and the fluorescence measures of those events stained with all different combinations of monoclonal antibodies conjugated to fluorochromes contained in a panel used to stain different replicate aliquots of the same sample are stored in a single data file.

25. The method of claim 1, wherein information from two distinct data files is merged directly without any correction.

26. The method of claim 1, wherein information from two different data files is merged after adjusting a relative position of populations of cellular events measured according to pre-established standards.

27. The method of claim 1, wherein the pre-established standards are reference microparticles.

28. The method of claim 27, wherein population of reference microparticles is uniform.

29. The method of claim 27, wherein a population of reference microparticles is composed of multiple populations of microbeads of differing size, density, volume, shape, amount of fluorescence, adhesion characteristics or other physico-chemical properties.

30. The method of claim 27, wherein a population of microparticles is composed of fluorescent particles.

31. The method of claim 27, wherein a population of microparticles is composed of microparticles whose surface is covered with anti-immunoglobulin antibodies.

32. The method of claim 27, wherein a population of microparticles is composed of a mixture of fluorescent microparticles whose surface is covered with anti-immunoglobulin antibodies.

33. The method of claim 27, wherein the microparticles are added in known numbers.



34. The method of claim 1, wherein serial dilutions of events from a data file corresponding to a neoplastic sample stained with a panel of multiple monoclonal antibody combinations into a data file containing information on the light scatter and fluorescence measures of cells contained in one or more normal/reactive samples stained with an identical panel of monoclonal antibodies, are made to evaluate the sensitivity at which a small number of neoplastic cells could be detected once diluted in normal/reactive cells at predefined known proportions.

35. The method of claim 1, wherein abnormal patterns of antigen expression related to cell activation and a cell function are detected.